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Average ovarian hormone levels, rather than daily values and their fluctuations, are related to facial preferences among women

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ABSTRACT

Hormones are of crucial importance for human behavior. Cyclical changes of ovarian hormones throughout women's menstrual cycle are suggested to underlie fluctuation in masculinity preference for both faces and bodies. In this study we tested this hypothesis based on daily measurements of estradiol and progesterone throughout menstrual cycle, and multiple measurements of women's preference towards masculinity of faces and bodies of men. We expected that due to a large variation among daily hormonal levels we would not observe a direct effect of daily hormone levels, but rather that average levels of ovarian hormones throughout the cycle (a reliable marker of a probability of conception) would better predict women's preferences. We found a negative relationship between average progesterone levels and facial masculinity preference, but only among women who were in long-term relationships. There was no relationship between facial masculinity preference and either of the estradiol or progesterone daily levels. Similarly, only average levels of hormones were significantly related to facial symmetry preference. For women who were in relationships estradiol was positively related to symmetry preference, while for single women this relationship was opposite. For body masculinity preference there were no significant relationships with neither averaged nor daily hormonal levels. Taken together, our results further suggest that overall cycle levels of ovarian hormones (averaged for a cycle) are better predictors of facial masculinity and symmetry preference than daily levels assessed during preferences' tests. Importantly, including information about relationship status in the investigations of hormonal bases of preferences is crucial.

1. Introduction

Hormones play an important role in human behavior and mating preferences. Women's levels of ovarian hormones (mainly progesterone and estradiol) are not constant, but change during their lifetime (being highest during peak reproductive years) and fluctuate throughout the menstrual cycle. Levels of hormones are under influence of many factors (Ellison, 2001; Jasienska, 2013), including genetics (Jasienska et al., 2006a; Jasienska et al., 2015) developmental conditions (Jasienska et al., 2006b; Jasienska et al., 2006c; Nunez-de la Mora et al., 2007), body size and shape (Jasienska et al., 2004; Ziomkiewicz et al., 2008) and lifestyle during adulthood (Jasienska & Ellison, 2004; Jasienska et al., 2006d). In fully functioning cycles, during the initial (follicular) phase of the cycle, a mature follicle is produced, which then releases an egg in a process called ovulation, allowing fertilization to occur. By the end of the follicular phase the ovary secretes high doses of estradiol. Mid-cycle drop in estradiol is a reasonable estimate of the day of ovulation (Lipson & Ellison, 1996). Following the ovulation, levels of estradiol still fluctuate, while levels of progesterone rise, preparing the endometrium for possible implantation of the fertilized egg. If implantation does not occur or if the embryo is not able to send hormonal signals to maternal organism, progesterone levels drop and menstrual bleeding starts.

It is hypothesized that women's preferences also fluctuate throughout the menstrual cycle due to hormonal changes, ovulation and, consequently, conception probability. “The ovulatory shift” hypothesis states that women around ovulation (when estradiol levels are high and progesterone levels are low) prefer partners with genes beneficial for their offspring, rather than partners providing high paternal investment. By contrast, during the low conception probability phase (i.e. luteal phase) women should prefer partners providing paternal investment and parenting skills. Therefore, women should express (*i*) increased masculinity preference around ovulation when searching for good quality in partner, and (*ii*) lowered masculinity preference in non-

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fertile phase when body is preparing for pregnancy and preference shifts towards social or economic support (Johnston et al., 2001). A number of articles testing this hypothesis have been published (for meta-analyses see (Gildersleeve et al., 2014; Wood et al., 2014), see also (Gildersleeve et al., 2013)), however a common agreement on the occurrence of such shifts is still missing, as some studies found shifts in masculinity preferences (Penton-Voak et al., 1999; Puts, 2005; Little et al., 2008), while other did not (Harris, 2012; Marcinkowska et al., 2016; Peters et al., 2009).

One of cues to good genes is exaggerated sexual dimorphism. Masculinity was suggested to be related to robust immune system, overall good health (Rhodes et al., 2003), strength (Fink et al., 2007), dominance (Geniole et al., 2015), increased reproductive success (Apicella et al., 2007) and men's anatomy (and related to this masculinity) shows evidence of adaptive design for contest competition (Puts, 2005). It has also been found that muscular men obtain higher attractiveness rating, than lean or heavily-set men (Dixson et al., 2014).

Nevertheless, exaggerated sexual dimorphism in men is also related to negative traits, such as dominant demeanor and aggressive behavior. Moreover, highly masculine men were found to be more sexually open (Boothroyd et al., 2008). Hence, the overall women's preference for masculinity is a trade-off between obtaining genetically “high quality” partner and paternal investment. Based on this trade-off in preferences for exaggerated sexual dimorphism in men it has been suggested that women's preferences should vary accordingly to the conception probability. In this study we test this hypothesis, by monitoring relationship of women's preferences for both facial and body masculinity to their daily ovarian hormones levels during multiple meetings throughout menstrual cycle.

We hypothesize that due to large variation in women's daily hormonal levels (Jasienska & Jasienski, 2008) and high irregularity of the natural, healthy cycles we would not observe a direct effect of hormone levels during a particular day of the cycle on the masculinity preference during the same day. Nonetheless, we propose that average levels of hormones throughout the cycle (a reliable marker of a probability of conception) should be a better predictor of women's preferences. We also hypothesize that in ovulatory cycles effects of hormone levels on preferences should be stronger than in non-ovulatory cycles (Roney & Simmons, 2008).

2. Methods

2.1. Participants

Women from the region of Malopolska in Poland were recruited personally or via mailing lists. Inclusion criteria for participation in the study were as follows: regular menstrual cycles (difference between lengths of consecutive cycles less than ± 5 days), no diabetes, no medically diagnosed problems of reproductive system, and no pregnancy, breast-feeding or use of hormonal contraception for a time period of at least 3 months prior to participation in the study. All participants provided a written consent. Out of 110 recruited participants 105 completed the study and 102 provided daily saliva samples collected throughout the entire menstrual cycle. All women completed a socio-demographic survey providing information on their age, past use of hormonal contraception, menstrual cycles, and reproductive (pregnancies and lactation) history. Participants also filled questionnaires on sexual orientation (on a 7 point Kinsey Scale, (Kinsey et al., 1948/1998)), relationship status and relationship satisfaction, partner's attractiveness and masculinity (on a 7 point Likert scale). Participants were between 21 and 37 years of age (Mean = 30, SD = 4.7), and 71 of them were in a long-term relationship (defined as longer than 6 months). Eight participants scored 4 or higher on Kinsey Sexual Orientation Scale (self-defined themselves as bi- or homo-sexual and, because the sexual orientation influences sexual preferences (Glassenberg et al., 2010), were excluded from further analysis. Women, on average, judged their partners to be attractive (Mean = 5.86, SD = 1.20) and masculine (Mean = 5.99, SD = 0.85), and overall relationship satisfaction was high (Mean = 5.92, SD = 1.20).

2.2. Procedure

All participants were asked to collect daily saliva samples each morning throughout one full menstrual cycle. All women were instructed about proper saliva collection and storing, and received written instructions and a set of 2 ml centrifuge tubes. Participants were also given 10 Lutenizing Hormone (LH) Ovulation Kits together with urine cups and written instruction. LH tests were individually conducted by each participant from 10th until 20th day of the cycle (days of the cycle with highest probability of ovulation occurrence) or until obtaining a positive result.

To account for hormonal variation throughout the cycle and possible caveats mentioned in previous studies, multiple testing sessions in different cycle phases were scheduled for each participant. All women attended 3 meetings throughout the cycle: the first meeting took place in early follicular phase (between 2nd and 8th day), the second meeting around ovulation (not later than 72 h after the positive result of ovulation detected by LH test or on 20th day of the cycle if there was no positive result) and the third meeting about one week after the ovulation (in mid-luteal phase).

During each meeting women chose via 2-alternative forced choice participants from 10 pairs of male faces varying in masculinity and from 3 pairs of male torsos. Visual stimuli constituted of 20 facial pictures of Caucasian men (aged 18–24). All faces were transformed with the PSYCHOMORPH Programme (Tiddeman et al., 2001) on a femininity–masculinity scale (Little et al., 2011). Two versions of each of the 20 original faces were created by adding or subtracting 50% of the linear difference between age matched 40 adult-male composite and a 40 adult-female composite. Thanks to such technique of facial transformation two stimuli pictures showed simultaneously to participants differed only in sexually dimorphic cues of face shape (DeBruine et al., 2010). Same technique was used for creation of torso visual stimuli (Little et al., 2007; Little et al., 2011). Visual stimuli were presented in a randomized order and location (left- or right-hand side of the screen).

2.3. Hormonal samples and measurements

Per each participant, hormonal profiles of 17-β-estradiol (E2) and progesterone (P) were obtained based on 15 daily measurements centered around the ovulation for E2 and the last 14 days of the cycle for P. Saliva samples were collected at least 30 min after eating, drinking or smoking and were frozen immediately after collection. All saliva samples were transported in portable freezers to the laboratory. Hormonal measurements were conducted using commercially available hormonal assays of DRG International Incl.: Elisa plates SLV4188 for 17-β-estradiol (sensitivity: 0.4 pg/ml, standard range: 1–100 pg/ml) and SLV3140 for 17-α-hydroxy-progesterone (sensitivity: 2.5 pg/ml, standard range: 10–5000 pg/ml). To achieve high standard of the measurements, all hormonal assays were conducted in duplicates. Quality of measurements was controlled for each plate separately by including (in duplicates) samples of known concentrations (“pools”) of P and E2 (in total these control measurements consisted of 19 pools per plate). Average levels of P for a cycle ranged from 17.2 to 416.3 pg/ml (mean = 113.53 pg/ml, SD = 70.88) and average levels of E2 ranged from 0.9 to 32.1 pg/ml (mean = 7.96 pg/ml, SD = 5.20). For P, inter-assay CV was 14.1%, and intra-assay was 4.9% and for E2, inter-assay CV was 10.01%, and intra-assay was 7.5%.